Proposed Registration Decision PRD2022-10

Beauveria bassiana strain R444 and Bb-Protec

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Overview

Proposed registration decision for *Beauveria bassiana* strain R444

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act*, is proposing registration for the sale and use of Beauveria bassiana strain R444 Technical, and Bb-Protec, containing the technical grade active ingredient *Beauveria bassiana* strain R444, to control twospotted spider mite and whitefly on a wide variety of greenhouse-grown ornamental and food crop plants, including plants grown for transplanting, and cannabis grown in greenhouses and enclosed structures.

An evaluation of available scientific information found that, under the approved conditions of use, the health and environmental risks and the value of the pest control products are acceptable.

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of *Beauveria bassiana* strain R444 and Bb-Protec.

What does Health Canada consider when making a registration decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment. These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the Health Canada regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides section of the Canada.ca website.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act.*

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (a) efficacy; (b) effect on host organisms in connection with which it is intended to be used; and (c) health, safety and environmental benefits and social and economic impact."

Before making a final registration decision on *Beauveria bassiana* strain R444 and Bb-Protec, Health Canada's PMRA will consider any comments received from the public in response to this consultation document.[3] Health Canada will then publish a Registration Decision[4] on *Beauveria bassiana* strain R444 and Bb-Protec, which will include the decision, the reasons for it, a summary of comments received on the proposed registration decision and Health Canada's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

What is *Beauveria bassiana* strain R444?

Beauveria bassiana is a fungus that grows naturally in soils throughout the world. It is a generalist entomopathogenic fungus that causes a disease in many types of insects that is often fatal. *Beauveria bassiana* strain R444 was isolated from soil from the Clanwilliam region of South Africa. When *Beauveria bassiana* strain R444 spores encounter an insect, they attach to, germinate, and penetrate the insect. Once inside the insect, the fungus grows and multiplies, resulting in death of the insect by internal tissue destruction. *Beauveria bassiana* strain R444 is used to control twospotted spider mite and whitefly on a wide variety of greenhouse-grown ornamental and food crop plants, including plants grown for transplanting, and cannabis grown in greenhouses and enclosed structures.

Health considerations

Can approved uses of *Beauveria bassiana* strain R444 affect human health?

Beauveria bassiana strain R444 is unlikely to affect your health when Bb-Protec is used according to the label directions.

Potential exposure to *Beauveria bassiana* strain R444 may occur when handling and applying Bb-Protec. When assessing health risks, several key factors are considered:

- the microorganism's biological properties (for example, production of toxic by-products);
- reports of any adverse incidents;
- its potential to cause disease or toxicity as determined in toxicological studies; and
- the level to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism.

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3 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

4 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

The levels used to assess risks are established to protect the most sensitive human population (for example, children and nursing mothers). As such, sex and gender are taken into account in the risk assessment. Only uses that are determined as having no health risks of concern are considered acceptable for registration.

Studies in laboratory animals describe potential health effects from large doses of exposure to a microorganism and identify any pathogenicity, infectivity and toxicity concerns.

When Beauveria bassiana strain R444 Technical and Bb-Protec were tested on laboratory animals, there was low toxicity following oral, pulmonary instillation, inhalation and dermal exposures. Bb-Protec is non-irritating to the skin. In the absence of eye irritation testing, the technical grade active ingredient and end-use product are assumed to be eye irritants. Furthermore, there was no sign that the microbial pest control agent, *Beauveria bassiana* strain R444, caused any disease.

All microorganisms, including *Beauveria bassiana* strain R444, contain substances that are potential sensitizers and thus, sensitivity may possibly develop in individuals exposed to potentially large quantities of *Beauveria bassiana* strain R444.

Residues in water and food

Dietary risks from food and water are acceptable.

Residues of *Beauveria bassiana* strain R444 on treated crops are possible at the time of harvest. However, no signs of infectivity or toxicity were observed when *Beauveria bassiana* strain R444 was tested on laboratory animals, and secondary metabolites are not expected to occur on edible portions of the crops above naturally occurring levels. In addition, the likelihood of *Beauveria bassiana* strain R444 contaminating drinking water is expected to be low as the label has the necessary mitigation measures to limit contamination of drinking water from the proposed uses of Bb-Protec. Consequently, dietary risks are acceptable.

Occupational risks from handling Bb-Protec

Occupational risks are acceptable when Bb-Protec is used according to label directions, which include protective measures.

Workers handling Bb-Protec can be exposed to *Beauveria bassiana* strain R444 through direct skin or eye contact or through inhalation. To protect workers from exposure to Bb-Protec, the label states that workers must wear personal protective equipment, including long-sleeved shirt, long pants, protective eyewear (goggles), waterproof gloves, socks and shoes and a NIOSH-approved particulate filtering facepiece respirator with any N, R or P filter. The product label includes measures to restrict access to the treated area for 4 hours or until sprays have settled. The occupational risks are acceptable when the precautionary statements on the label are observed.

Risks in residential and other non-occupational environments

Estimated risk for non-occupational exposure is acceptable.

Bb-Protec is proposed for commercial use as a diluted broadcast spray in greenhouses and enclosed structures only. Residential and non-occupational exposure to Bb-Protec is therefore expected to be low when label directions are observed. Consequently, the risk to residents and the general public is acceptable.

Environmental considerations

What happens when *Beauveria bassiana* strain R444 is introduced into the environment?

Environmental risks are acceptable.

Beauveria bassiana is a common microorganism that is widely distributed in the natural environment. It is an insect pathogen that causes white muscardine disease in insects. When spores of the fungus come into contact with a susceptible host insect, they germinate, enter the body of the insect and grow, eventually killing the insect. The species is typically identified as a soil microorganism. However, it can be found on insects and plants. Minimal environmental exposure to *Beauveria bassiana* strain R444 is expected following the proposed use in greenhouses and enclosed structures.

The applicant submitted studies designed to examine the effects of *Beauveria bassiana* strain R444 to birds, bees, terrestrial arthropods, fish, and aquatic arthropods. No adverse effects to birds or fish were observed during testing. Bb-Protec was toxic to bees, parasitic wasps, ladybird beetle larvae, and daphnids. Test results suggested pathogenicity to parasitic wasps, ladybird beetle larvae, and bees. As a result, the end-use product label will contain statements identifying the potential for harm to beneficial arthropods and that applications must avoid direct contact to foraging bees. The end-use product label will also contain standard statements restricting users from activities that may release *Beauveria bassiana* strain R444 into aquatic environments from its use in greenhouses and enclosed structures. Exposure to outdoor environments is expected to be minimal since the use is restricted to greenhouses and enclosed structures, therefore, significant adverse effects to non-target organisms are not expected.

Based on a critical review of data and published scientific literature submitted by the applicant, the risks to birds, wild mammals, fish, non-target terrestrial and aquatic arthropods, terrestrial and aquatic non-arthropod invertebrates, and plants are acceptable when Bb-Protec is applied according to directions on the label.

Value considerations

What is the value of Bb-Protec?

Bb-Protec is a biological insecticide that provides control of twospotted spider mite and whitefly on a wide variety of greenhouse-grown ornamental and food crop plants, including plants grown for transplanting, and cannabis grown in greenhouses and enclosed structures.

Bb-Protec will provide users with a new strain of *Beauveria bassiana* for control of two economically important greenhouse pests, twospotted spider mite and whitefly. Due to the complex mode of action of this product, resistance is unlikely to occur and therefore Bb-Protec may aid with the management of resistance of the target pests against other insecticides.

Measures to minimize risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the labels of Beauveria bassiana strain R444 Technical and the end-use product, Bb-Protec, to address the potential risks identified in this assessment are as follows.

Key risk-reduction measures

Human health

The signal words ‘POTENTIAL SENSITIZER’ and ‘CAUTION EYE IRRITANT’ will appear on the primary display panel of the labels. Also, workers handling or applying Bb-Protec must wear a long-sleeved shirt, long pants, protective eyewear (goggles), waterproof gloves, socks and shoes and a NIOSH-approved particulate filtering facepiece respirator with any N, R or P filter. Furthermore, all unprotected workers are restricted from entering treated areas during application and for 4 hours following application or until sprays have settled.

Environment

The end-use product label will include environmental precaution statements to reduce the potential for contamination of aquatic systems from the use of Bb-Protec. The label for the end-use product will also include an environmental precaution statement to minimize the risk to beneficial insects and pollinators used in greenhouse integrated pest management programs.

Next steps

Before making a final registration decision on *Beauveria bassiana* strain R444 and Bb-Protec, Health Canada's PMRA will consider any comments received from the public in response to this consultation document. Health Canada will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). Health Canada will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed decision and Health Canada's response to these comments.

Other information

When the Health Canada makes its registration decision, it will publish a Registration Decision on *Beauveria bassiana* strain R444 and Bb-Protec (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room. For more information, please contact the PMRA's Pest Management Information Service.

Science evaluation

Beauveria bassiana strain R444 and Bb-Protec

1.0 The active ingredient, its properties and uses

1.1 Identity of the active ingredient

Active microorganism	*Beauveria bassiana* strain R444
Function	Mycoinsecticide – for the control of whitefly and twospotted spider mite
Binomial name	*Beauveria bassiana* strain R444
Taxonomic designation[1]	
Superkingdom	Eukaryota
Kingdom	Fungi
Subkingdom	Dikarya
Phylum	Ascomycota
Class	Sordariomycetes
Order	Hypocreales
Family	Cordycipitaceae
Genus	*Beauveria*
Species	*bassiana*
Strain	R444
Patent Status information	None
Minimum purity of active	Technical grade active ingredient: 8.84×10^9 spores/g Bb-Protec End-use Product: 1×10^8 colony-forming units (CFU)/g
Identity of relevant impurities of toxicological and/or environmental significance	The technical grade active ingredient does not contain any impurities or microcontaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards. Beauvericin, a secondary metabolite of *B. bassiana* strain R444, has been detected in the technical grade active ingredient, but is below the limit of quantification (0.5 ppm) in the end-use product. These levels of beauvericin suggest that this strain is not likely a hyper-producer of this metabolite.

[1] National Center for Biotechnology Information - Taxonomy Browser (https://www.ncbi.nlm.nih.gov/taxonomy)

1.2 Physical and chemical properties of the end-use product

End-use product–Bb-Protec

Property	Result
Colour	White
Physical State	Powder
Odour	No odour
Viscosity (mPa at 20°C)	n/a
pH (1%)	6.5
Relative Density	0.58 g/mL

1.3 Directions for use

Bb-Protec controls twospotted spider mite and/or whitefly on a wide variety of greenhouse-grown ornamental and food crop plants, including plants grown for transplanting, and cannabis grown in greenhouses and enclosed structures. Bb-Protec is applied as a foliar spray at a rate of 0.5 to 1.0 g product per L water, applied to runoff with a re-application interval of 3 to 7 days. As Bb-Protec is a live fungal organism, applications of fungicides should be avoided 3 days before and following application of this product. For details, refer to Appendix I, Table 4.

1.4 Mode of action

Beauveria bassiana strain R444 is a soil fungus that causes white muscardine disease in various insects. When *Beauveria bassiana* strain R444 spores encounter a susceptible insect, they attach to, germinate and penetrate through the cuticle. Once inside the insect, the fungus grows and multiplies while also overcoming the host response and immune defense reactions. Death of the insect is caused by internal tissue destruction. *Beauveria bassiana* strain R444 is classified by the Insecticide Resistance Action Committee as a Class UNF (Fungal Agents of Unknown or Uncertain Mode of Action) Insecticide.

2.0 Methods of analysis

2.1 Methods for identification of the microorganism

Acceptable methodologies for detection, isolation and enumeration of the active ingredient, *Beauveria bassiana* strain R444, were submitted by the applicant. *Beauveria bassiana* strain R444 has been fully characterized with respect to the origin of strain, natural occurrence and biological properties. *Beauveria bassiana* strain R444 can be identified to the species level by sequencing of the internal transcribed spacer (ITS) ribosomal deoxyribonucleic acid (rDNA), and can be identified to the strain level by microsatellite and multi-locus sequencing.

2.2 Methods for establishment of purity of seed stock

Beauveria bassiana strain R444 has been deposited into the South African National Fungal Culture Collection (deposit number 7861) and the German Collection of Microorganisms and Cell Cultures (DSMZ) (deposit number DSMZ 32777). The strain is maintained in an acceptable manner in order to maintain purity, viability, and genetic stability.

2.3 Methods to define the content of the microorganism in the manufactured material used for the production of formulated product

The guarantees of the technical grade active ingredient and the end-use product are expressed in units of spores/g and colony forming units (CFU)/g, respectively. Representative data on five batches of the technical grade active ingredient and five batches of the end-use product were submitted. The methods for determining the concentration and viability of spores, and for determining CFU counts were adequately described.

2.4 Methods to determine and quantify residues (viable or non-viable) of the active microorganism and relevant metabolites

As noted above, acceptable methods are available to enumerate the microorganism and to distinguish this microbial pest control agent (MPCA) from other strains of *Beauveria bassiana*. An acceptable method to quantify the level of beauvericin in the technical grade active ingredient and in the end-use product was described.

2.5 Methods for determination of relevant impurities in the manufactured material

The quality assurance procedures used to limit contaminating microorganisms during the manufacture of Beauveria bassiana strain R444 Technical and Bb-Protec are acceptable. These procedures include sterilization of all equipment and media as well as sampling of the culture media, stock cultures and production batches for purity and contamination.

The absence of human pathogens and below-threshold levels of contaminating microorganisms were shown in the microbial screening of batches of Bb-Protec using standard microbiological methods as well as quantitative polymerase chain reaction for detecting and enumerating microbial contaminants of concern. All batches of Bb-Protec conform to the limits set out in the Organisation for Economic Co-operation and Development (OECD) issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43].

2.6 Methods to determine storage stability, shelf-life of the microorganism

Storage stability data were provided for Bb-Protec. Results support a storage period of 6 months at 4°C.

3.0 Impact on human and animal health

3.1 Toxicity and infectivity summary

3.1.1 Testing

A detailed review of the submitted toxicological studies was conducted in support of the technical grade active ingredient, Beauveria bassiana strain R444 Technical, and the associated end-use product, Bb-Protec.

To address the health hazard requirements for Beauveria bassiana strain R444 Technical and Bb-Protec, the applicant submitted acute oral toxicity/infectivity, acute pulmonary toxicity/pathogenicity, dermal toxicity and dermal irritation studies. These studies were performed with the end-use product, Bb-Protec. The applicant also submitted an acute inhalation toxicity study performed with ground spores of *B. bassiana* strain R444 which was equivalent to Beauveria bassiana strain R444 Technical.

In the acute oral infectivity and toxicity study, ~75 day old Sprague Dawley rats (12/sex) were each administered a single oral dose of Bb-Protec in deionized water (10% w/v). Each rat received a nominal dose of 1.25×10^7 CFU of *B. bassiana* strain R444 in a 1.0 mL volume. Animals were observed for up to 21 days with interim sacrifices on Days 3, 7, and 14. There were no mortalities and no-treatment related effects. The MPCA was undetectable in sacrificed animals by Day 21, thus establishing a pattern of clearance.

In the acute pulmonary infectivity and toxicity study, 60 day old Sprague Dawley rats (15/sex) were exposed by the intratracheal route to Bb-Protec in deionized water (10% w/v). Each rat received a nominal dose of 1.25×10^6 CFU in a volume of 0.1 mL. Animals were then observed for up to 21 days with interim sacrifices on Days 0, 3, 7, and 14. There were no mortalities and no treatment related effects. The MPCA was undetectable in sacrificed animals by Day 14, thus establishing a pattern of clearance.

In the acute dermal toxicity study, 7–8 week old Sprague-Dawley rats (5/sex) were dermally exposed to 5050 mg/kg body weight (bw) of Bb-Protec (containing *B. bassiana* strain R444 at 1.25×10^8 CFU/g) in deionized water (1 g/mL) for 24 hours to an area of approximately 10% of body surface area. Following exposure, the animals were observed for a period of 14 days. There were no mortalities. One female lost 7 g body weight between Days 0 and 7, but gained weight between Days 7 and 14. Very slight erythema was observed in two females on Day 4 that resolved by Day 7.

In the primary dermal irritation study, young adult albino New Zealand White rabbits (2 male, 1 female) were dermally exposed to 500 mg of Bb-Protec (containing *B. bassiana* strain R444 at 1.25×10^8 CFU/g) in 0.5 mL deionized water for four hours to a 5 × 5 cm site. Animals were then observed for 72 hours. Irritation was scored by the method of Draize. There were no signs of irritation or other effects in any animal. The maximum average score (MAS) was 0.0/8.0.

In the acute inhalation toxicity study, 9-10 week old Sprague-Dawley rats (5/sex) were exposed by nose-only inhalation to mechanically ground spores of *B. bassiana* strain R444 (5×10^9 spores/g) for four hours at a concentration of 3.22 mg/L. Animals were then observed for 14 days. There were no mortalities. There was discolouration of the lungs in two males and one female. All animals lost weight between Days 0 and 1; two females also lost weight between Days 1 and 3. All body weight effects were reversible.

Test results are summarized in Appendix I, Tables 1 and 2.

3.1.2 Additional information

The applicant submitted a scientific rationale to waive the requirement for intraperitoneal infectivity testing on Beauveria bassiana strain R444 Technical. The rationale was found to be acceptable based on the fact that no infectivity/pathogenicity was observed in acute oral and acute pulmonary infectivity/pathogenicity testing with *B. bassiana* strain R444 (contained in Bb-Protec). No further data are required for intraperitoneal infectivity testing on Beauveria bassiana strain R444 Technical.

A survey of published literature performed by PMRA found that, in rare cases, *B. bassiana* has the potential to act as an opportunistic pathogen. These incidents, however, are associated with compromised immune systems or a history of surgery/injury. This survey of published literature also found evidence that some isolates of *B. bassiana* possess IgE reactive proteins and thus could potentially elicit sensitization reactions. There are also several reports of allergenicity in workers handling *B. bassiana* conidia and therefore, the respiratory route is of particular concern. In addition, some strains of *B. bassiana* are known to produce the secondary metabolites beauvericin, bassianolide, oosporein, bassiacridin, bassianin and tenellin. A 5-batch analysis of the technical grade active ingredient using acceptable methods confirmed the presence of beauvericin. However, a 5-batch analysis of the end-use product was also conducted and the presence of beauvericin was not detected above the limit of quantification (LOQ) of 0.5 ppm.

The applicant submitted a document (Report date: November 1, 2018) under the subject line ‘Reporting of Hypersensitivity Incidence’ stating that no incidence of adverse effects among researchers, employees or growers involved in the manufacture, handling or application of *B. bassiana* strain R444 had been reported.

3.1.3 Incident reports related to human and animal health

Beauveria bassiana strain R444 is a new active ingredient pending registration for use in Canada, and as of 25 April 2022, no incident reports had been submitted to the PMRA.

3.1.4 Hazard analysis

The data package submitted in support of registering Beauveria bassiana strain R444 Technical and Bb-Protec was reviewed from the viewpoint of human health and safety and was determined to be acceptable.

Bb-Protec is of low toxicity by the oral, pulmonary and dermal routes, and is not pathogenic or infective by the oral and pulmonary routes. Ground spores of *Beauveria bassiana* strain R444 is of low toxicity via the inhalation route. Bb-Protec is not irritating to the skin. Because eye irritation testing was not conducted, the technical grade active ingredient and end-use product labels will include the standard PPE for eye protection, and the hazard statements 'CAUTION: EYE IRRITANT'. No reports of hypersensitivity or allergenicity incidents among workers were reported for this strain. However, all MPCAs are considered potential sensitizers and, therefore, the hazard statement "POTENTIAL SENSITIZER" will appear on the principal display panel of the technical grade active ingredient and end-use product labels. The statements "May cause sensitization. May irritate eyes. Avoid contact with skin, eyes or clothing." is also required on the secondary panel of the technical grade active ingredient and end-use product labels under the "PRECAUTIONS" section.

Higher tier subchronic and chronic toxicity studies were not required because Tier I studies: a) did not indicate the end-use product to be acutely toxic by the oral, dermal or pulmonary route of administration or the technical grade active ingredient to be acutely toxic by the inhalation route of administration; and b) there were no indications of any infectivity or pathogenicity in any animals tested with the MPCA.

Within the available scientific literature, there are no reports that suggest *B. bassiana* has the potential to cause adverse effects on the endocrine system of animals. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for this MPCA.

3.2 Occupational, residential and bystander risk assessment

3.2.1 Occupational and post-application exposure and risk

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for handlers, mixer/loaders, and applicators exists. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe was a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Beauveria bassiana* has not frequently been identified as a dermal wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Furthermore, end-use product testing with Bb-Protec showed low toxicity and no infectivity via the oral and pulmonary routes, and low toxicity via the dermal route. Testing with spores of *B. bassiana* strain R444 showed low toxicity via the inhalation route. Hazard testing with the end-use product also showed that Bb-Protec is not irritating to the skin. In lieu of testing, the PMRA considers all microorganisms as ocular irritants; therefore, the end-use product and technical grade active ingredient may cause eye irritation. The PMRA also assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions.

Risk mitigation measures, such as personal protective equipment (PPE), including a long-sleeved shirt, long pants, protective eyewear (goggles), waterproof gloves, socks and shoes and a NIOSH-approved particulate filtering facepiece respirator with any N, R or P filter are required to minimize exposure and protect handlers, mixer/loaders and applicators. Furthermore, all unprotected workers are prohibited from entering treated areas where Bb-Protec has been applied for 4 hours or until sprays have settled.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of Bb-Protec. Overall, occupational risks to workers are acceptable when the precautionary statements on the labels are followed which include PPE.

3.2.2 Residential and bystander exposure and risk

Bb-Protec is proposed for use only in greenhouses and enclosed structures. This use is not expected to result in significant residential and bystander exposure due to drift. Bystander exposure will be mitigated by the inclusion of a statement on the label requiring all unprotected workers to remain out of treated areas until sprays have dried. Also, the technical grade active ingredient and end-use product are of low toxicity and there were no signs that the MPCA, *B. bassiana* strain R444, caused any disease in studies on laboratory animals. Consequently, the health risks to bystanders and individuals in residential areas are acceptable.

3.3 Dietary exposure and risk assessment

3.3.1 Food

While the proposed use pattern may result in dietary exposure with possible residues in or on agricultural commodities, the risks from consuming crops treated with Bb-Protec are acceptable because Bb-Protec, containing *Beauveria bassiana* strain R444, demonstrated no toxicity, pathogenicity or infectivity in Tier I studies.

In an analysis of the end-use product, beauvericin (a secondary metabolite known to be produced by this MPCA) was not detected above the LOQ of 0.5 ppm. Application of Bb-Protec is not expected to increase levels of beauvericin on treated crops beyond existing levels produced by naturally-occurring fungi.

If toxic secondary metabolites are produced by the MPCA in insects, their occurrence in edible food commodities would be negligible due to common hygiene practices and standards that prevent insect parts from being contained in foodstuff.

When the end-use product is applied to cannabis grown in greenhouses and enclosed structures, the health risk from consumer exposure is acceptable.

Consequently, there is no health risk to the general population, including infants and children, or animals.

3.3.2 Drinking water

Dietary exposure from drinking water is expected to be low as the label has the necessary mitigation measures to limit contamination of drinking water from the proposed uses in greenhouses and enclosed structures. The label will instruct users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal and to not allow effluent or runoff from greenhouses and enclosed structures containing this product to enter lakes, streams, ponds or other waters. Municipal treatment of drinking water is also expected to further reduce the transfer of residues to drinking water. Furthermore, *B. bassiana* strain R444 demonstrated no toxicity, pathogenicity or infectivity in Tier I studies. Health risks from residues of *B. bassiana* strain R444 in drinking water are acceptable due to the low toxicity/pathogenicity profiles of *Beauveria bassiana* strain R444 and Bb-Protec, and limited exposure following application of the end-use product.

3.3.3 Acute and chronic dietary risks for sensitive subpopulations

Calculations of acute reference doses and acceptable daily intakes are not usually possible for predicting acute and long-term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (in other words, no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that *B. bassiana* strain R444 is of low oral toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to this MPCA than the general population. Thus, there are no threshold effects of concern and, as a result, there is no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of this MPCA, including neurological effects from pre- or post-natal exposures, and cumulative effects on infants and children of the MPCA and other registered microorganisms that have a common mechanism of toxicity, does not apply to this MPCA. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *B. bassiana* strain R444 to human health.

3.3.4 Aggregate exposure and risk

Aggregate exposure is the total exposure to a single pesticide that may occur from food, drinking water, residential and other non-occupational sources, and from all known or plausible exposure routes (oral, dermal and inhalation).

In an aggregate risk assessment, the combined potential risk associated with food, drinking water and various residential exposure pathways is assessed. A major consideration is the likelihood of co-occurrence of exposures. Additionally, only exposures from routes that share common toxicological endpoints can be aggregated.

Bb-Protec is considered to be of low toxicity by the oral, pulmonary and dermal routes and the end-use product will not be applied near or to drinking water. Furthermore, non-occupational exposure will be low when Bb-Protec is used as directed on the label. When the end-use product is used as labelled, there is reasonable certainty that no harm will result from aggregate exposure of residues of *B. bassiana* strain R444.

3.3.5 Maximum residue limits

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain in or on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally specified as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada specifies science-based MRLs to ensure the food Canadians eat is safe.

Residues of *B. bassiana* strain R444 on treated food crops are possible at the time of harvest. Dietary risk to humans from the proposed use of Bb-Protec is acceptable due to the low toxicity profile of *B. bassiana* strain R444 and that application of Bb-Protec is not expected to increase levels of beauvericin on treated crops beyond existing levels produced by naturally-occurring fungi.

In addition, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Therefore, the PMRA has determined that specification of an MRL under the *Pest Control Products Act* is not required for *B. bassiana* strain R444.

3.4 Cumulative assessment

The *Pest Control Products Act* requires that the PMRA consider the cumulative exposure to pesticides with a common mechanism of toxicity. In its assessment of common mechanism of toxicity, the PMRA considers both the taxonomy of MPCAs and the production of any potentially toxic metabolites. For the current evaluation, the PMRA has determined that *B. bassiana* strain R444 shares a common mechanism of toxicity with other strains of *B. bassiana* that are used as MPCAs; *B. bassiana* strain ANT-03, *B. bassiana* strain PPRI 5339, *B. bassiana* strain GHA, *B. bassiana* strain CFL-A, and *B. bassiana* strain HF23.

The potential health risks from cumulative exposure of *B. bassiana* strain R444 and these other MPCAs are acceptable when used as labelled given their low toxicity and pathogenicity. As well, application of Bb-Protec is not expected to increase levels of beauvericin on treated crops beyond existing levels produced by naturally-occurring fungi.

4.0 Impact on the environment

4.1 Fate and behavior in the environment

No studies were submitted to address the environmental fate and behaviour of *B. bassiana* strain R444; however, environmental fate data (Tier II/III) are not normally required at Tier I, and are only triggered if significant toxicological effects in non-target organisms are noted in Tier I testing.

Beauveria bassiana is a generalist entomopathogen that causes white muscardine disease in insects of various orders. When spores of the fungus come into contact with an insect host, they germinate, enter the body of the insect and grow hyphae, eventually killing the insect. Although the fungus is a generalist, individual strains may be specific to select species. Isolates of this species can be naturally found on all continents with the exception of Antarctica. In Canada, isolates of this species can be found in all provinces. While this species is typically identified as a soil microorganism, conidia of *B. bassiana* can also be naturally found on insects and plants. There are also reports of some isolates of *B. bassiana* that demonstrate the ability to colonize plants endophytically.

The proposed uses of Bb-Protec are limited to greenhouses and enclosed structures. The intended applications are foliar. Although the uses preclude any direct exposure to outdoor environments, exposure to *B. bassiana* strain R444 may occur through human activity such as composting of plant waste and water management practices. Afterwards, the dispersal of *B. bassiana* strain R444 should be limited to the movement of treated plant materials, natural vectors (for example, insects), and to a limited extent, runoff.

In the event that *B. bassiana* strain R444 does reach outdoor soil environments, the organism is expected to behave as it would in nature. As a ubiquitous soil microorganism, it is likely that *B. bassiana* would settle in the soil where it is commonly found, rather than percolate through soil. Therefore, mobility through the soil is expected to be minimal. Evidence suggests that *B. bassiana* strain R444 is able to survive in soil, if certain environmental conditions (for example, moisture, pH) are met, but that, over time, populations would be expected to return to naturally occurring levels.

Overall, the proposed use in greenhouses and enclosed structures of Bb-Protec is not expected to significantly increase natural populations of *B. bassiana* in outdoor terrestrial or aquatic environments.

4.2 Effects on non-target species

PMRA has a four-tiered approach to environmental testing of microbial pesticides. Tier I studies consist of acute studies on up to seven broad taxonomic groups of non-target organisms exposed to a maximum hazard or Maximum Challenge Concentration of the MPCA. The Maximum Challenge Concentration is generally derived from the amount of the MPCA, or its toxin, expected to be available following application at the maximum recommended label rate

multiplied by a safety factor. Tier II studies consist of environmental fate (persistence and dispersal) studies, as well as additional acute toxicity testing of MPCAs. Tier III studies consist of chronic toxicity studies (life cycle studies), as well as definitive toxicity testing (for example, LC_{50}, LD_{50}). Tier IV studies consist of experimental field studies on toxicity and fate, and are required to determine whether adverse effects are realized under actual use conditions.

The type of environmental risk assessment conducted on MPCAs varies depending on the tier level that was triggered during testing. For many MPCAs, Tier I studies are sufficient to conduct environmental risk assessments. Tier I studies are designed to represent "worst-case" scenarios where the exposure conditions greatly exceed the expected environmental concentrations. The absence of adverse effects in Tier I studies are interpreted as minimal risk to the group of non-target organisms. However, higher tiered studies will be triggered if significant adverse effects on non-target organisms are identified in Tier I studies. These studies provide additional information that allows the PMRA to refine the environmental risk assessments. In the absence of adequate environmental fate and/or field studies, a screening level risk assessment can be performed to determine if the MPCA is likely to pose a risk to a group of non-target organisms.

The screening level risk assessment uses simple methods, conservative exposure scenarios (for example, direct application at a maximum application rate) and sensitive toxicity endpoints. A risk quotient (RQ) is calculated by dividing the exposure estimate by an appropriate toxicity value (RQ = exposure/toxicity), and the risk quotient is then compared to the level of concern (level of concern = 1 for most species, 0.4 for acute risk to pollinators, and 2 for glass plate studies using the standard beneficial arthropod test species, *Typhlodromus pyri* and *Aphidius rhopalosiphi*; level of concern = 1 is used for higher tier tests of the standard arthropod test species and for other arthropod test species).

If the screening level risk quotient is below the level of concern, the risk is considered negligible and no further risk characterization is necessary. If the screening level risk quotient is equal to or greater than the level of concern, then a refined risk assessment is performed to further characterize the risk. A refined assessment takes into consideration more realistic exposure scenarios (environmental fate and/or field testing results). Refinements may include further characterization of risk based on exposure modelling, monitoring data, results from field or mesocosm studies, and probabilistic risk assessment methods. Refinements to the risk assessment may continue until the risk is adequately characterized or no further refinements are possible.

4.2.1 Effects on terrestrial organisms

A detailed review of the terrestrial non-target studies and other supporting information was conducted in support of the technical grade active ingredient, Beauveria bassiana strain R444 Technical, and the end-use product, Bb-Protec.

Six studies were submitted to address the hazards of Bb-Protec to birds, honeybees, ladybird beetles, green lacewings, and parasitic wasps. These studies were performed with Bb-Protec. Data submitted under human and animal health toxicity testing were considered to assess the risk of harm to wild mammals. Acceptable scientific information was also provided to address the hazard to terrestrial plants.

The acute oral toxicity/pathogenicity of Bb-Protec to 23-day-old bobwhite quail (*Colinus virginianus*) was assessed over 30 days. Bb-Protec was administered to birds (3 replicates of 10), as a suspension in deionised water, by oral gavage at a nominal concentration of 5.29×10^7 CFU/kg bw daily for five consecutive days. There were no signs of toxicity or pathogenicity in any treated animal for the duration of the study.

In a 30-day dietary toxicity study, 30 honeybees *(Apis mellifera)* were exposed overnight to Bb-Protec in the diet (50% w/v sugar suspension) at a measured concentration of 3.33×10^6 CFU/mL. Mortality in the untreated control, attenuated test item, and 3.33×10^6 CFU/mL groups on Day 30 was 0, 24, and 17%, respectively. Based on the average consumption of feed, bees in the attenuated test item and treatment groups consumed the equivalent of 1.33×10^4 CFU/bee and 1.07×10^4 CFU/bee, respectively. There was, however, uncertainty regarding the MPCA enumeration in the feeding suspension, and the product was not consumed by bees in one replicate chamber in the attenuated test item group. Furthermore, exposure to the feeding solutions was overnight only and an alternate food source (pollen) was presented concurrently with the test suspension. Humidity levels were not reported, but need to be sufficiently high to allow for MPCA germination in order to assess pathogenicity.

In a 27-day contact toxicity study, 30 honeybees *(A. mellifera)* were exposed to Bb-Protec in deionised water by topical application of a measured dose of 9.5×10^3 CFU/bee. There were no treatment-related differences in cumulative mortality; however, mortality in the active test substance group occurred earlier (Day 15) than the attenuated test item (Day 20) and negative control (Day 21), suggesting pathogenicity. There appeared to be more abnormal behavior in the attenuated test item group, relative to both the control and the test item groups.

In a 10-day dietary toxicity/pathogenicity study, ladybird beetle larvae (*Lindorus (Rhyzobius) lopanthae*) (30/group) were exposed to Bb-Protec at measured concentrations of 2.18×10^6, 1.70×10^5, 1.76×10^4, 1.76×10^3, and 4.36×10^2 CFU/g diet. Mortality in the negative control, attenuated test item, and 2.18×10^6, 1.70×10^5, 1.76×10^4, 1.76×10^3, and 4.36×10^2 CFU/g diet groups on Day 10 was 20, 43, 40, 40, 30, 17, and 30%, respectively. Fungal growth was observed on Day 10 for 3 of the 12 and 9 of the 12 dead larvae found in the 1.70×10^5 and 2.18×10^6 CFU/g diet treatment groups, respectively. Due to low humidity, adverse effects on ladybird beetles may be underestimated.

In a 10-day dietary toxicity/pathogenicity study, green lacewing larvae (*Chrysoperla rufilabris)* (30/group) were exposed to Bb-Protec at measured concentrations of 1.64×10^6, 3.09×10^5, 1.70×10^4, 2.67×10^3, and 1.94×10^2 CFU/g diet. There were no treatment-related effects. However, due to low humidity, effects in green lacewing cannot be ruled out.

In a 10-day dietary toxicity/pathogenicity study, parasitic wasps (*Pediobius foveolatus)* (25-35/group) were exposed to Bb-Protec in 30% sugar water at measured concentrations of 8.16×10^5, 5.00×10^4, 1.45×10^4, 2.10×10^3, and 4.96×10^2 CFU/mL diet. Mortality in the negative control, attenuated test item, and 8.16×10^5, 5.00×10^4, 1.45×10^4, 2.10×10^3, and 4.96×10^2 CFU/mL diet groups on Day 10 was 16, 25, 46, 44, 23, 19, and 15% respectively. The higher mortality in the 8.16×10^5 and 5.00×10^4 CFU/mL diet groups compared to the attenuated test substance group suggests pathogenicity. Fungal growth was observed on 3 of the 42 and 3 of the 35 dead wasps found in the 8.16×10^5 and 5.00×10^4 CFU/mL diet treatment groups, respectively, at study termination. Due to low humidity, adverse effects in parasitic wasps may be underestimated.

Beauveria bassiana strain R444 is not considered to be a mammalian pathogen, and the data submitted (see Section 3.1 under Impact on Human and Animal Health) demonstrated that Bb-Protec is of low toxicity by the oral, pulmonary and dermal routes, and is not pathogenic or infective by the oral and pulmonary routes. *Beauveria bassiana* strain R444 is not toxic via the inhalation route. Bb-Protec is not irritating to the skin. No additional data or information to characterize the hazard to wild mammals are required.

A waiver for non-target terrestrial plant testing was not submitted. However, in efficacy trials of Bb-Protec, there were no reports of phytoxicity or crop injury. *Beauveria bassiana* strain R444 also does not appear on authoritative lists of plant pathogens and pests. Therefore, the proposed uses of Bb-Protec are not expected to result in adverse effects to non-target terrestrial plants. No additional data or information to characterize the hazard to non-target terrestrial plants are required.

In addition to the above information, no reports of adverse effects to birds, non-arthropod invertebrates or plants were found in the PubMed database. Adverse effects to various terrestrial arthropods are described in published scientific literature. These findings were expected since the MPCA is an entomopathogen.

Based on all the available data and information on the effects of Bb-Protec to non-target terrestrial organisms, the risks to birds, wild mammals, arthropods (including honeybees), non-arthropod invertebrates, plants, and other microorganisms from the proposed use in greenhouses and enclosed structures of Bb-Protec are acceptable with the proposed precautionary labelling statements.

Results from non-target arthropod testing and the known entomopathogenic nature of *B. bassiana* strain R444 indicate that certain non-target arthropod species may be adversely affected in the event of direct exposure to Bb-Protec, particularly when maximum application rates and repeat intervals are followed. Precautionary measures are required on the Bb-Protec label to alert operators of the potential hazard to beneficial arthropods that may be used in greenhouse Integrated Pest Management programs. Furthermore, precautionary labelling will state that Bb-Protec may be harmful to beneficial arthropods, and that direct contact with beneficial arthropods should be avoided.

Test results are summarized in Appendix I, Table 3.

4.2.2 Effects on aquatic organisms

A detailed review of the aquatic non-target studies and other supporting information was conducted in support of the technical grade active ingredient, Beauveria bassiana strain R444 Technical, and the end-use product, Bb-Protec.

Four studies were submitted to address the hazards of Bb-Protec to fish and aquatic arthropods. These studies were performed with the end-use product, Bb-Protec.

In a 96-hour toxicity study, zebrafish (*Danio rerio*) (7/group) were aquatically exposed to Bb-Protec under semi-static renewal conditions at nominal concentrations of 125, 250, 500, 1000, and 2000 mg/L (equivalent to 1.7×10^3, 9.3×10^3, 3×10^3, 7×10^3, and 8.7×10^4 CFU/mL, measured). There were no treatment-related effects. Even though the study was scientifically valid, this study was of limited utility in the risk assessment since zebrafish are a warm water species and the study was conducted at 22-24°C, which is not reflective of Canadian environmental conditions.

In a 30-day toxicity/pathogenicity/infectivity study, 10 rainbow trout (*Onchorynchus mykiss*) were exposed to Bb-Protec under static renewal conditions. Exposure was both aquatic (3.98×10^3 CFU/mL media) and dietary (1.53×10^3 CFU/g feed). There were no treatment-related effects. Even though the study was scientifically valid, this study did not entirely meet PMRA requirements and was of limited use in the risk assessment due to an inadequate number of test fish.

In a 48-hour toxicity study, daphnids (*Daphnia magna*) (20/group) were aquatically exposed to Bb-Protec under static conditions at nominal concentrations of 62.5, 125, 250, 500, 1000, and 2000 mg/L (equivalent to 3×10^2, 7×10^2, 3.3×10^3, 3×10^3, 3×10^3, and 3×10^3 CFU/mL, measured). There were no treatment-related effects. Inconsistencies were noted in viability testing of the dosing solutions.

In a 21-day toxicity/pathogenicity study, 50 daphnids (*Daphnia magna*) were aquatically exposed to Bb-Protec at 4.67×10^4 CFU/mL under static renewal conditions. Mortality in the negative control, active test item, attenuated test item, and sterile filtrate groups on Day 5 was 0, 100, 44, and 2%, respectively. The observed mortality appeared to be due to toxicity, not pathogenicity, since onset of mortality was similar between the active and attenuated test item groups, and deaths occurred early in the observation period. The total number of live offspring per surviving adult, the production rate of first brood, and mean surviving adult body length were significantly reduced in the attenuated test item relative to the untreated control. Exposure to the sterile filtrate also resulted in a significant reduction in mean surviving adult body length compared to the media control. An oily surface was observed in the test item, attenuated test item and test item sterile filtrate treatments, along with clumping of daphnids at the media surface. Exposure to aquatic organisms from applications of the end-use product in greenhouses and enclosed structures is expected to be negligible, and therefore the risk is acceptable.

The applicant did not submit a request to waive the requirement for non-target aquatic plants and algae testing. However, there was a lack of phytotoxicity or crop injury reported in any of the efficacy trials conducted for these submissions. As well, there are no reports of phytotoxicity or plant pathogenicity reported in the published scientific literature from *B. bassiana* (from a search of PubMed using keywords 'Beauveria and phytotoxicity' and 'Beauveria and plant and pathogenicity'). The proposed uses of Bb-Protec are not expected to result in adverse effects to non-target aquatic plants and algae. No additional data or information to characterize the hazard to non-target aquatic plants and algae are required.

Test results are summarized in Appendix I, Table 3.

Based on all the available data and information on the effects of Bb-Protec to non-target aquatic organisms, and the anticipated minimal environmental exposure resulting from the uses of Bb-Protec in greenhouses and enclosed structures, the risks to fish, aquatic arthropods, plants and algae are acceptable.

4.3 Incident reports related to the environment

Beauveria bassiana strain R444 is a new active ingredient pending registration for use in Canada, and as of 25 April 2022, no incident reports had been submitted to the PMRA.

5.0 Value

Value information reviewed in support of the label claims included efficacy trials, use history information from South Africa, and scientific rationales to extrapolate from crops tested in the efficacy trials to all labelled host crop claims. The efficacy trials included eight greenhouse trials on greenhouse whitefly or sweetpotato whitefly, and seven trials on twospotted spider mite. Trials were conducted on various greenhouse crops, field tomatoes, and field strawberries. The efficacy trials demonstrated control of these pests when applied at a rate of 0.5 to 1.0 g product per L water. Based on the nature of the product (entomopathogenic fungus) and the results of the efficacy trials, a re-application interval of 3 to 7 days is reasonable to provide control of the listed pests.

Whitefly and twospotted spider mite are widespread pests of greenhouse-grown crops. As a result, there are many alternative pesticides registered for control of these pests in most Insecticide Resistance Action Committee mode of action groups. In addition, several other strains of *Beauveria bassiana* (strains ANT-03, GHA, and PPRI 5339) are also registered for use against these pests in greenhouse vegetables, greenhouse ornamentals, and cannabis grown in greenhouses and enclosed structures.

Bb-Protec may contribute to resistance management of twospotted spider mite and whitefly. Bb-Protec is an entomopathogenic soil fungus and has a complex mode of action; therefore, resistance is unlikely to occur. Bb-Protec may also contribute to risk reduction by reducing reliance on chemical alternatives.

Like many biological pesticides, death does not occur immediately following application of Bb-Protec, and multiple applications may be necessary to achieve full control. Bb-Protec should be applied under high humidity and low UV light conditions, and use of broad-spectrum fungicides should be avoided for at least three days before and after application of the product.

While no phytotoxic effects were observed in any of the submitted trials, Bb-Protec is labelled for application to a large variety of crops, including ornamentals, of which many were not tested for crop tolerance. As a result, a label statement stating that Bb-Protec has not been tested on all crops or varieties of crops and recommending the user tests the product on a sample plot before widespread application is present on the label.

The reviewed value information supports a claim of control of whitefly and two-spotted spider mite on listed greenhouse food and ornamental crops and cannabis grown in greenhouses and enclosed structures, when applied at a rate of 0.5 to 1.0 g product per L spray, applied to runoff, with a re-application interval of 3 to 7 days. For details, refer to Appendix I, Table 4.

6.0 Pest control product policy considerations

6.1 Toxic substances management policy considerations

The Toxic Substances Management Policy (TSMP) is a federal government policy developed to provide direction on the management of substances of concern that are released into the environment. The TSMP calls for the virtual elimination of Track 1 substances, in other words, those that meet all four criteria outlined in the policy: persistent (in air, soil, water and/or sediment), bio-accumulative, primarily a result of human activity and toxic as defined by the *Canadian Environmental Protection Act*. The *Pest Control Products Act* requires that the TSMP be given effect in evaluating the risks of a product

During the review process, Beauveria bassiana strain R444 Technical and Bb-Protec were assessed in accordance with the PMRA Regulatory Directive DIR99-03[5] and evaluated against the Track 1 criteria. The PMRA has reached the conclusion that Beauveria bassiana strain R444 Technical and Bb-Protec do not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.

[5] Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*.

6.2 Formulants and Contaminants of Health or Environmental Concern

During the review process, contaminants in the technical as well as formulants and contaminants in the end-use product are compared against Parts 1 and 3 of the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern.*[6] The list is used as described in the PMRA Notice of Intent NOI2005-01[7] and is based on existing policies and regulations, including the Toxic Substances Management Policy and Formulants Policy[8] and taking into consideration the Ozone-depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

- Technical grade product Beauveria bassiana strain R444 Technical and the end-use product, Bb-Protec, do not contain any formulants or contaminants identified in the *List of Pest Control Product Formulants of Health or Environmental Concern*.

The use of formulants in registered pest control products is assessed on an ongoing basis through PMRA formulant initiatives and Regulatory Directive DIR2006-02.

7.0 Proposed regulatory decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act*, is proposing registration for the sale and use of Beauveria bassiana strain R444 Technical, and Bb-Protec, containing the technical grade active ingredient *Beauveria bassiana* strain R444, to control twospotted spider mite and whitefly on a wide variety of greenhouse-grown ornamental and food crop plants, including plants grown for transplanting, and cannabis grown in greenhouses and enclosed structures.

An evaluation of available scientific information found that, under the approved conditions of use, the health and environmental risks and the value of the pest control products are acceptable.

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6 SI/2005-114, last amended on 25 June 2008. See Justice Laws website, Consolidated Regulations, List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern.

7 PMRA's Notice of Intent NOI2005-01, *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern under the New Pest Control Product Act*.

8 DIR2006-02, *Formulants Policy and Implementation Guidance Document*.

List of abbreviations

°C	degree(s) Celsius
%	percent
bw	body weight
cm	centimetre(s)
CFU	colony forming units
d	day
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures)
EP	end-use product
g	gram(s)
h	hour(s)
IgE	immunoglobulin E
IRAC	Insecticide Resistance Action Committee
ITS	internal transcribed spacer
kg	kilogram(s)
L	litre(s)
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOEC	lowest observed effect concentration
LOQ	level of quantification
MAS	maximum average score
mL	millilitre(s)
mg	milligram(s)
MPCA	microbial pest control agent
MRL	maximum residue limit
n/a	not applicable
NIOSH	National Institute for Occupational Safety & Health
NOEC	no observed effect concentration
NOEL	no observed effect level
OECD	Organisation for Economic Co-operation and Development
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
ppm	parts per million
rDNA	ribosomal deoxyribonucleic acid
RQ	risk quotient
TGAI	technical grade of the active ingredient
TSMP	Toxic Substances Management Policy
UV	ultra violet
w/v	weight/volume

Appendix I Tables and figures

Table 1 Toxicity profile of Bb-Protec

(Effects are known or assumed to occur in both sexes unless otherwise noted; in such cases, sex-specific effects are separated by semi-colons)

Study Type/ Animal/ PMRA#	Study Results
21-day Acute Oral Infectivity/Toxicity Sprague Dawley rat PMRA No. 2971929	Acute oral LD_{50} >1.25×10^{7} CFU/rat. **LOW Toxicity** and not infective or pathogenic via oral gavage.
21-day Acute Pulmonary Instillation Infectivity/Toxicity Sprague Dawley rat PMRA No. 2971931	Acute pulmonary LD_{50} >1.25×10^{6} CFU/rat. **LOW Toxicity** and not infective or pathogenic via intratracheal instillation.
14-day Acute Dermal Toxicity Sprague Dawley rat PMRA No. 3111964	Acute dermal LD_{50} >5050 mg/kg bw. **LOW Toxicity**
72-hour Dermal Irritation New Zealand white rabbit PMRA No. 2971936	Non-irritating to the skin (MAS 0.0/8.0).

Table 2 Toxicity profile of *B. bassiana* strain R444

(Effects are known or assumed to occur in both sexes unless otherwise noted; in such cases, sex-specific effects are separated by semi-colons)

Study Type/ Animal/ PMRA#	Study Results
14-day Acute Inhalation Toxicity[1] Sprague Dawley rat PMRA No. 3237643	Acute inhalation LC_{50}>3.22 mg/L air (Limit Test). **LOW Toxicity** via nose-only inhalation.

[1]The test substance was ground spores of *B. bassiana* strain R444, which is considered equivalent to Beauveria bassiana strain R444 Technical.

Table 3 Toxicity/Pathogenicity of Bb-Protec to non-target species

Organism	Exposure	Significant Effect, Comments	Reference (PMRA #)
Terrestrial Organisms			
Vertebrates			
Birds			
Bobwhite Quail (*Colinus virginianus*), 23-day-old	Oral– 5.29×10^7 CFU/kg bw/day for 5 days	There were no mortalities or signs of pathogenicity observed. 30-d LD_{50} >5.29×10^7 CFU/kg bw/day over 5 days **LOW TOXICITY** **NOT PATHOGENIC**	2971940
Invertebrates			
Arthropods			
Honeybees (*Apis mellifera*), young adult worker	Dietary– 3.33×10^6 CFU/mL in 50% w/v sugar/water	Study ended on Day 30 There were significant increases in cumulative mortality in the test item and attenuated test item groups. One replicate chamber in the attenuated test item group did not feed on the treatment. Exposure to the feeding solutions was overnight only. Humidity level was not reported. 30-d LD_{50} >1.07×10^4 CFU/bee	2971945

Organism	Exposure	Significant Effect, Comments	Reference (PMRA #)
		30-d NOEC < 1.07×10^4 CFU/bee **TOXIC** **PATHOGENICITY COULD NOT BE ASSESSED**	
Honeybees (*Apis mellifera*), young adult worker	Contact–9.5×10^3 CFU/bee in deionised water	Study was terminated on Day 27 when mortality in control group exceeded 20%. Mortality occurred considerably earlier in the test item group compared to the attenuated test item. Abnormal behaviour was observed in the attenuated test item group. 27-d LD_{50} >9.5×10^3 CFU/bee 27-d NOEL (attenuated test item: behaviour) <9.5×10^3 CFU/bee **LOW TOXICITY** **POSSIBLE PATHOGENICITY**	2971946
Ladybird beetle (*Lindorus (Rhyzobius) lopanthae*), larvae	Dietary–2.18×10^6, 1.70×10^5, 1.76×10^4, 1.76×10^3, and 4.36×10^2 CFU/g diet	Mortality in the negative control, attenuated test item, and 2.18×10^6, 1.70×10^5, 1.76×10^4, 1.76×10^3, and 4.36×10^2 CFU/g diet groups on Day 10 was 20, 43, 40, 40, 30, 17, and 30%, respectively at Day 10. Fungal growth was observed on 3/12 and 9/dead larvae found in the 2.18×10^6 and 1.70×10^5 CFU/g diet groups, respectively. 10-day LC_{50} > 2.18×10^6 CFU/g diet 10-d NOEC (mortality, fungal growth) = 1.76×10^4 CFU/g diet **TOXIC** **POSSIBLE PATHOGENICITY**	3130257
Green lacewing (*Chrysoperla rufilabris)*, larvae	Dietary–1.64×10^6, 3.09×10^5, 1.70×10^4, 2.67×10^3, and 1.94×10^2 CFU/g diet	There were no adverse effects on survival or pupation. Humidity level was lower than required for optimal germination. 10-day LC_{50} >1.64×10^6 CFU/g diet	3130258

Organism	Exposure	Significant Effect, Comments	Reference (PMRA #)
		LOW TOXICITY **PATHOGENICITY COULD NOT BE ASSESSED**	
Parasitic wasp (*Pediobius foveolatus)*, newly emerged adults	Dietary–8.16×10^5, 5.00×10^4, 1.45×10^4, 2.10×10^3, and 4.96×10^2 CFU/mL diet in 30% w/v sugar/water	There were significant increases in cumulative mortality in the attenuated test item, 8.16×10^5 CFU/mL, and 5.00×10^4 CFU/mL diet groups. Fungal growth was observed on 3/35 and 3/42 dead wasps found in the 5.00×10^4 CFU/mL and 8.16×10^5 CFU/mL diet treatment groups, respectively. 10-day LC_{50} >8.16×10^5 CFU/mL diet 10-d NOEC (mortality, fungal growth) = 1.45×10^4 CFU/mL diet **TOXIC** **POSSIBLE PATHOGENICITY**	3130259
Aquatic Organisms			
Vertebrates			
Fish			
Zebrafish (*Danio rerio*)	Aquatic–1.7×10^3, 9.3×10^3, 3×10^3, 7×10^3, and 8.7×10^4 CFU/mL media (measured)	There were no treatment-related effects. Inconsistencies were noted in viability testing of the dosing solutions. 96-h LC_{50} >8.7×10^4 CFU/mL **LOW TOXICITY** This study is of limited utility due to testing of a warm water fish species at 22–24°C, which is not reflective of Canadian environmental conditions.	2971942
Rainbow trout (*Oncorhynchus mykiss*), female	Aquatic–3.98×10^3 CFU/mL media Dietary–1.53×10^3 CFU/g feed	There were no treatment-related toxicity, infectivity, or pathogenicity effects observed. 30-d aquatic LC_{50} >3.98×10^3 CFU/mL media 30-d dietary LC_{50} >1.53×10^3 CFU/g diet	3130255

Organism	Exposure	Significant Effect, Comments	Reference (PMRA #)
		LOW TOXICITY **NO INFECTIVITY** **NO PATHOGENICITY** This study is of limited utility due to an inadequate number of test subjects.	
Invertebrates			
Arthropods			
Daphnids (*Daphnia magna*), juvenile	Aquatic– 4.67 × 10^4 CFU/mL media	Mortality in the negative control, active test item, attenuated test item, and sterile filtrate groups on Day 5 was 0, 100, 44, and 2%, respectively. Mortality appeared due to toxicity, not pathogenicity, since onset of mortality was similar between the active and attenuated test item groups and deaths occurred early in the observation period. Total number of live offspring, production rate of first brood, and surviving adult body length were significantly reduced in the attenuated test item group. Surviving adult body length was significantly reduced in the sterile filtrate group. An oily surface and clumping of daphnids at the media surface was observed in all of the test item, attenuated test item, and sterile filtrate groups. 5-d LC_{50} <4.67 × 10^4 CFU/mL 21-d LOEC (attenuated test item: reproduction, body length) <4.67 × 10^4 CFU/mL **TOXIC** **NOT PATHOGENIC**	3130261

Organism	Exposure	Significant Effect, Comments	Reference (PMRA #)
Daphnids (*Daphnia magna*), juvenile	Aquatic– 3×10^2, 7×10^2, 3.3×10^3, 3×10^3, 3×10^3, and 3×10^3 CFU/mL media	There were no effects on mortality/immobilization. Inconsistencies were noted in viability testing of the dosing solutions. 48-h LC_{50} >3×10^3 CFU/mL **LOW TOXICITY**	2971951

Table 4 List of supported uses

Supported label claim for Bb-Protect, *Beauveria bassiana* Strain R444
Greenhouse-grown Cannabis: For control of whitefly and twospotted spider mite, apply 0.5 to 1.0 g product per mL spray to runoff. Re-apply at 3 to 7 day intervals.
Greenhouse crop plants and plants grown for transplanting **Ornamentals Grown in Greenhouse or in Protected Environments**: Subgroup 2A (container grown flowering annuals and perennials; Subgroup 2B (container grown annuals, perennials for cut flowers): For control of whitefly and twospotted spider mite, apply 0.5 to 1.0 g product per mL spray to runoff. Re-apply at 3 to 7 day intervals.
Greenhouse crop plants and plants grown for transplanting **Fruiting Vegetables (Crop Group 8-09):** African eggplant, bell pepper, bush tomato, cocona, currant tomato, eggplant, garden huckleberry, goji berry, groundcherry, martynia, naranjilla, non-bell pepper, okra, pea eggplant, pepino, roselle, scarlet eggplant, sunberry, tomatillo, tomato, tree tomato, and cultivars, varieties and hybrids of these: For control of whitefly, apply 0.5 to 1.0 g product per mL spray to runoff. Re-apply at 3 to 7 day intervals.
Greenhouse crop plants and plants grown for transplanting **Cucurbit Vegetables (Crop Group 9):** chayote (fruit), Chinese waxgourd (Chinese preserving melon), citron melon, cucumber, gherkin, gourd (edible, includes Chinese okra, cucuzza, hechima, hyotan), Momordica spp. (includes balsam apple, balsam pear, bitter melon, Chinese cucumber), muskmelon (includes cantaloupe, casaba, crenshaw melon, golden pershaw melon, honey balls, honeydew melon, mango melon, Persian melon, pineapple melon, Santa Claus melon, snake melon, true cantaloupe), pumpkin, summer squash (includes crookneck squash, scallop squash, straightneck squash, vegetable marrow, zucchini), watermelon, winter squash (includes acorn squash, butternut squash, calabaza, hubbard squash, spaghetti squash): For control of whitefly, apply 0.5 to 1.0 g product per mL spray to runoff. Re-apply at 3 to 7 day

Supported label claim for Bb-Protect, *Beauveria bassiana* Strain R444
intervals.
Greenhouse crop plants and plants grown for transplanting **Leafy Vegetables (Crop Group 4-13)**: Abyssinian cabbage, arugula, bitter lettuce, blackjack, broccoli raab, cat's whiskers, cham-chwi, cham-na-mul, chervil (fresh leaves), Chinese amaranth, Chinese broccoli, Chinese cabbage (bok choy), Chinese violet, chipilin, cilantro (fresh leaves), collards, corn salad, cosmos, dandelion, dang-gwi, dillweed (fresh leaves), dock, dol-nam-mul, ebolo, endive, escarole, English primrose, fameflower, feather cockscomb, garden cress, garden purslane, garland chrysanthemum, good King Henry, Hanover salad, head lettuce, huauzontle, jute leaves, kale, Indian aster, leaf lettuce, leafy amaranth, maca, Malabar spinach, mizuna, mustard greens, New Zealand spinach, orach, parsley (fresh leaves), plantain (including buckhorn plantain and common plantain), radicchio (red chicory), radish leaves, rape greens, seakale cabbage, shepherd's purse, spinach, Swiss chard, tanier spinach, tree spinach, turnip greens, upland cress, watercress, wild rocket, winter purslane, and cultivars, varieties and hybrids of these: For control of whitefly, apply 0.5 to 1.0 g product per mL spray to runoff. Re-apply at 3 to 7 day intervals.
Greenhouse crop plants and plants grown for transplanting **Brassica Head and Stem Vegetables (Crop Group 5-13)**: broccoli, Brussels sprouts, cabbage, Chinese cabbage (napa), cauliflower, and cultivars, varieties and hybrids of these: For control of whitefly, apply 0.5 to 1.0 g product per mL spray to runoff. Re-apply at 3 to 7 day intervals.
Greenhouse GROWN FOR TRANSPLANT ONLY: Berries (Crop Group 13-07): Blackberry, Blueberry (highbush), Blueberry (Lowbush), black current, red current, Elderberry, Gooseberry, Huckleberry, Black and red raspberry, Amur river berry, Aronia berry, Bayberry, Bearberry, Bilberry, Buffalo current, Buffaloberry, Che, Chilean guava, Chokecherry, Cranberry, European barberry, Grape, Highbush cranberry, Edible honeysuckle, Jostaberry, Juneberry, Fuzzy Kiwifruit, Hardy Kiwifruit, Lingonberry, Maypop, Mountain pepper berry, Mulberry, Muntries, Native current, Partridgeberry, Phalsa, Pincherry, Riberry, Salal, Schisandra berry, Sea buckthorn, Serviceberry, Strawberry, Wild raspberry and cultivars, varieties and hybrids of these: For control of whitefly and twospotted spider mite, apply 0.5 to 1.0 g product per mL spray to runoff. Re-apply at 3 to 7 day intervals.
Greenhouse Strawberry For control of whitefly and twospotted spider mite, apply 0.5 to 1.0 g product per mL spray to runoff. Re-apply at 3 to 7 day intervals.
Greenhouse crop plants and plants grown for transplanting **Stalk, Stem, and Leaf Petioles (Crop Group 22):** agave, aloe vera, asparagus, bamboo shoots, cardoon, celery, Chinese celery, Florence fennel (fresh leaves and stalk), fern (edible fiddlehead, including black lady fern, bracken fern, broad buckler fern, cinnamon fern, lady fern, leather fern, mother fern, ostrich fern, vegetable fern, zenmai fern), fuki, kohlrabi, palm hearts, prickly pear, rhubarb, sea kale, Texas prickly pear, udo, zuiki, and cultivars, varieties and hybrids of these.

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For control of whitefly, apply 0.5 to 1.0 g product per mL spray to runoff. Re-apply at 3 to 7 day intervals.
Greenhouse crop plants and plants grown for transplanting **Herbs and Spices (Crop Group 19):** allspice, angelica, anise (seed), annatto, balm (lemon balm), basil, black caraway, borage, burnet, camomile, caper buds, caraway, cardamom, cassia bark, cassia buds, catnip, celery seed, chervil (dried), Chinese chive, chive, cinnamon, clary, clove buds, cilantro(leaf), coriander (cilantro seed), costmary, culantro (leaf), culantro (seed), cumin, curry (leaf), dill (dillweed), dill (seed), fennel, fenugreek, Florence fennel (seed), grains of paradise, horehound, hyssop, juniper berry, lavender, lemongrass, lovage (leaf), lovage (seed), mace, marigold, marjoram (includes sweet or annual marjoram, wild marjoram or oregano and pot marjoram), mustard (seed), nasturtium, nutmeg, parsley (dried), pennyroyal, pepper, poppy (seed), rosemary, rue, saffron, sage, savory, star anise, sweet bay (bay leaf), tansy, tarragon, thyme, vanilla, wintergreen, woodruff, wormwood: For control of whitefly and twospotted spider mite, apply 0.5 to 1.0 g product per mL spray to runoff. Re-apply at 3 to 7 day intervals.

References

A. List of studies/Information submitted by the registrant

1.0 The active substance, its properties and uses

PMRA Document Number	Reference
2971885	2018, M 2.7.1 Origin, Deviation and Identification of the MPCA, DACO: M2.7.1 CBI
2971886	2018, M 2.7.1 Appendix 1, DACO: M2.7.1 CBI
2971887	2018, M 2.7.2 Biological Properties of the MPCA, DACO: M2.7.2 CBI
2971888	Bidochka, M. J., Kasperski, J. E., & Wild, G. A. (1998). Occurrence of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* in soils from temperate and near-northern habitats. *Canadian Journal of Botany, 76*(7), 1198-1204, DACO: M2.7.2 CBI
2971889	Bing, L. A., & Lewis, L. C. (1993). Occurrence of the entomopathogen *Beauveria bassiana* (Balsamo) Vuillemin in different tillage regimes and in *Zea mays* L. and virulence towards *Ostrinia nubilalis* (Hübner). *Agriculture, ecosystems & environment*, *45*(1-2), 147-156, DACO: M2.7.2
2971891	Gatarayiha, M. C., Laing, M. D., & Miller, R. M. (2010). Effects of adjuvant and conidial concentration on the efficacy of *Beauveria bassiana* for the control of the two spotted spider mite, *Tetranychus urticae*. *Experimental and Applied Acarology*, *50*(3), 217-229, DACO: M2.7.2, M9.8.1
2971894	Goble, T. A., Conlong, D. E., & Hill, M. P. (2015). Virulence of *Beauveria brongniartii* and *B. bassiana* against *Schizonycha affinis* white grubs and adults (Coleoptera: S carabaeidae). *Journal of Applied Entomology*, *139*(1-2), 134-145, DACO: M2.7.2, M9.8.1
2971897	Ignoffo, C. M. (1992). Environmental factors affecting persistence of entomopathogens. *Florida entomologist*, 516-525, DACO: M2.7.2, M9.8.1
2971899	Mascarin, G. M., & Jaronski, S. T. (2016). The production and uses of *Beauveria bassiana* as a microbial insecticide. *World Journal of Microbiology and Biotechnology*, *32*(11), 1-26, DACO: M2.7.2, M9.2.1
2971900	Monzon, A. J., Guharay, F., & Klingen, I. (2008). Natural occurrence of *Beauveria bassiana* in *Hypothenemus hampei* (Coleoptera: Curculionidae) populations in unsprayed coffee fields. *Journal of Invertebrate Pathology*, *97*(2), 134-141, DACO: M2.7.2, M9.5.1, M9.8.1, M9.8.2
2971901	Mwamburi, L. A., Laing, M. D., & Miller, R. M. (2010). Laboratory screening of insecticidal activities of *Beauveria bassiana* and *Paecilomyces lilacinus* against larval and adult house fly (*Musca domestica* L.). *African Entomology*, *18*(1), 38-46, DACO: M2.7.2, M9.8.1
2971902	Oduor, G. I., Smith, S. M., Chandi, E. A., Karanja, L. W., Agano, J. O., & Moore, D. (2000). Occurrence of *Beauveria bassiana* on insect pests of stored maize in Kenya. *Journal of Stored Products Research*, *36*(2), 177-185, DACO: M2.7.2, M9.5.1, M9.8.1, M9.8.2

PMRA Document Number	Reference
2971903	Ortiz-Urquiza, A., & Keyhani, N. O. (2013). Action on the surface: entomopathogenic fungi versus the insect cuticle. *Insects*, *4*(3), 357-374, DACO: M2.7.2
2971904	de Oliveira, D. G. P., Lopes, R. B., Rezende, J. M., & Delalibera Jr, I. (2018). Increased tolerance of *Beauveria bassiana* and *Metarhizium anisopliae* conidia to high temperature provided by oil-based formulations. *Journal of Invertebrate Pathology*, *151*, 151-157, DACO: M2.7.2
2971905	St Leger, R. J., Allee, L. L., May, B., Staples, R. C., & Roberts, D. W. (1992). World-wide distribution of genetic variation among isolates of *Beauveria* spp. *Mycological Research*, *96*(12), 1007-1015, DACO: M2.7.2, M9.5.1, M9.8.1, M9.8.2
2971906	Zimmermann, G. (2007). Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Science and Technology*, *17*(6), 553-596, DACO: M2.7.2, M4.3.3, M9.5.1, M9.8.1, M9.8.2
2971907	2018, M 2.8 Manufacturing Methods and Quality Assurance, DACO: M2.8 CBI
2971917	2017, M 2.10.2 Appendix 1, DACO: M2.10.2 CBI
2971923	2018, Bb-Protec Determination of miscibility at 30C, DACO: M2.12 CBI
3093826	2020, M 2.9.2 Potency Estimation and Product Guarantee June 20th Deficiency Response, DACO: M2.9.2 CBI
3093827	2019, Detection and Enumeration *of Beauveria bassiana* strain R444 and microbial contamination in five batches of the MPCP Bb-Protec, DACO: M2.11, M2.9.2, M2.9.3 CBI
3137300	2020, M 2.7.1 Appendix 2 June 20th Deficiency Response, DACO: M2.10.1, M2.7.1 CBI
3137301	2020, M 2.7.1 Appendix 3 June 20th Deficiency Response, DACO: M2.10.1, M2.7.1 CBI
3137302	2019, M 2.7.1 Appendix 4 June 20th Deficiency Response, DACO: M2.10.1, M2.7.1 CBI
3137303	2020, M 2.7.1 Appendix 5 June 20th Deficiency Response, DACO: M2.10.1, M2.7.1 CBI
3137305	2020, M 2.7.1 Appendix 7 June 20th Deficiency Response, DACO: M2.10.1, M2.7.1 CBI
3137308	Goble, T. A., Costet, L., Robène, I., Nibouche, S., Rutherford, R. S., Conlong, D. E., & Hill, M. P. (2012). *Beauveria brongniartii* on white grubs attacking sugarcane in South Africa. *Journal of Invertebrate Pathology*, *111*(3), 225-236, DACO: M2.7.1
3137310	2020, M 2.7.2 Biological Properties of the MPCA: relationship discussion June 20th Deficiency Response, DACO: M2.7.2 CBI
3137311	Humber, R. A. (1992). Collection of entomopathogenic fungal cultures: catalog of strains. *Collection of entomopathogenic fungal cultures: catalog of strains*, DACO: M2.7.2

PMRA Document Number	Reference
3137319	2019, Determining the growth of *Beauveria bassiana* strain R444 at varying temperatures, DACO: M2.7.2 CBI
3237631	2020, Validation of the analytical method to determine the beauvericin content in the five batches of Bb-Protec, DACO: M2.7.2 CBI
3237632	2021, Validation of the analytical method to determine the Beauvericin content in the technical product *Beauveria bassiana* R444, DACO: M2.7.2 CBI
3237635	2021, Bb-Protec Production Process, DACO: M2.8 CBI
3237637	2021, TGAI 5 batch anaylsis, DACO: M2.9.2 CBI
3237638	2021, Certificate of Analysis Beauveria bassiana R444 TGAI, DACO: M2.9.2 CBI
3237640	2021, Internal Storage stability data for Eco-Bb (Bb-Protec) spores on substrate, DACO: M2.11 CBI
3237641	2021, Long Term Storage Stability Test on the microbial pest control product Bb-Protec, DACO: M2.11 CBI
3238306	2021, Determination of the beauvericin content in the five batches of technical Beauveria bassiana R444, DACO: M2.7.2 CBI
3283527	2021, M2.11 Storage Stability October 2021 Clarifications, DACO: M2.11 CBI
3283528	2021, Eco-Bb:Colony Forming Unit Protocol, DACO: M2.11 CBI
3283529	2021, M2.12 Summary of Physical and Chemical Properties October 2021 Clarifications, DACO: M2.12 CBI

2.0 Human and animal health

PMRA Document Number	Reference
2971926	2018, M 4.1 Summary, DACO: M4.1
2971927	2018, M 4.2.1 Infectivity and Toxicity Summary, DACO: M4.2.1
2971928	2018, M 4.2.2 Acute Oral Infectivity and Toxicity, DACO: M4.2.2
2971929	2018, Bb-Protec Acute Oral Toxicity/Pathogenicity in Rats, DACO: M4.2.2
2971930	2018, M 4.2.3 Acute Pulmonary Infectivity and Toxicity, DACO: M4.2.3
2971931	2018, Bb-Protec Acute Pulmonary Toxicity/Pathogenicity Study in Rats, DACO: M4.2.3
2971932	2018, M 4.3.1 Acute Infectivity Summary, DACO: M4.3.1
2971933	2018, M 4.3.3 Intraperitoneal Infectivity, DACO: M4.3.3
2971934	2018, M 4.5.1 Irritation Summary, DACO: M4.5.1
2971935	2018, M 4.5.2 Dermal Irritation Study, DACO: M4.5.2
2971936	2018, Bb-Protec Acute Dermal Irritation In Rabbits, DACO: M4.5.2
2971937	2018, M 4.6 Reporting of Hypersensitivity Incidence, DACO: M4.6
3111965	2020, M 4.9.1 Acute Inhalation Toxicity July 12th Deficiency Response, DACO: M4.9

PMRA Document Number	Reference
3111963	2020, M 4.4 Acute Dermal Toxicity July 12th Deficiency Response, DACO: M4.4
3111964	2020, Bb-Protec Acute Dermal Toxicity in Rats, DACO: M4.4
3111966	2019, Bb-Protec Acute Inhalation Toxicity in Rats, DACO: M4.9
3130250	2020, M 4.9.1 Acute Inhalation Toxicity July 12th TGAI Deficiency Response, DACO: M4.9 CBI
3130252	2019, Bb-Protec Acute Inhalation Toxicity in Rats, DACO: M4.9 CBI
3237642	2021, M 4.9.1 Acute Inhalation Study 2019-0781 August 2020 Deficiency Response, DACO: M4.9
3237643	2021, Bb-Protec Acute Inhalation Toxicity in Rats, DACO: M4.9, M7.0
3237644	2021, M 7 Food Residues, DACO: M7.0
3237647	Joshi, M., & Misra, A. (2001). Dry powder inhalation of liposomal Ketotifen fumarate: formulation and characterization. *International journal of pharmaceutics*, *223*(1-2), 15-27, DACO: M7.0
3237649	2019, Determining the growth of *Beauveria bassiana* strain R444 at varying temperatures, DACO: M7.0

3.0 Environment

PMRA Document Number	Reference
2971900	Monzon, A. J., Guharay, F., & Klingen, I. (2008). Natural occurrence of *Beauveria bassiana* in *Hypothenemus hampei* (Coleoptera: Curculionidae) populations in unsprayed coffee fields. *Journal of Invertebrate Pathology*, *97*(2), 134-141, DACO: M2.7.2, M9.5.1, M9.8.1, M9.8.2
2971902	Oduor, G. I., Smith, S. M., Chandi, E. A., Karanja, L. W., Agano, J. O., & Moore, D. (2000). Occurrence of *Beauveria bassiana* on insect pests of stored maize in Kenya. *Journal of Stored Products Research*, *36*(2), 177-185, DACO: M2.7.2, M9.5.1, M9.8.1, M9.8.2
2971905	St Leger, R. J., Allee, L. L., May, B., Staples, R. C., & Roberts, D. W. (1992). World-wide distribution of genetic variation among isolates of *Beauveria* spp. *Mycological Research*, *96*(12), 1007-1015, DACO: M2.7.2, M9.5.1, M9.8.1, M9.8.2
2971906	Zimmermann, G. (2007). Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Science and Technology*, *17*(6), 553-596, DACO: M2.7.2, M4.3.3, M9.5.1, M9.8.1, M9.8.2
2971940	2018, Bb-Protec Avian Oral Toxicity Microbial Limit Test with Bobwhite Quail, DACO: M9.2.1
2971942	2018, Bb-Protec Fish (*Danio rerio*) Acute Toxicity Test Final Report, DACO: M9.4.1
2971943	2019, M 9.5.1 Terrestrial Arthropods, DACO: M9.5.1

PMRA Document Number	Reference
2971945	2019, Effects of the Microbial Pest Control Product Bb-Protec on the honeybee, *Apis mellifera*, in a oral toxicity test, DACO: M9.5.1
2971946	2019, Effects of the Microbial Pest Control Product Bb-Protec on the honeybee, *Apis mellifera*, in a contact toxicity test, DACO: M9.5.1
2971947	Chandler, D., Davidson, G., & Jacobson, R. J. (2005). Laboratory and glasshouse evaluation of entomopathogenic fungi against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), on tomato, *Lycopersicon esculentum*. *Biocontrol Science and Technology*, *15*(1), 37-54, DACO: M9.5.1
2971948	Ullah, M. S., & Lim, U. T. (2017). Laboratory evaluation of the effect of *Beauveria bassiana* on the predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae). *Journal of Invertebrate Pathology*, *148*, 102-109, DACO: M9.5.1
2971949	Wu, S., Xing, Z., Sun, W., Xu, X., Meng, R., & Lei, Z. (2018). Effects of *Beauveria bassiana* on predation and behavior of the predatory mite *Phytoseiulus persimilis*. *Journal of invertebrate pathology*, *153*, 51-56, DACO: M9.5.1
2971951	2018, Bb-Protec *Daphnia magna* Acute Immobilization Test Final Report, DACO: M9.5.2
3130255	2020, Toxicity and pathogenicity of the Microbial Pest Control Product Bb-Protec to Rainbow Trout (*Oncorhynchus mykiss*) during a 30 day test, DACO: M9.4.1
3130257	2018, M 9.5.1 Appendix 1 July 12th TGAI Deficiency Response, DACO: M9.5.1
3130258	2018, M 9.4.1 Appendix 2 July 12th TGAI Deficiency Response, DACO: M9.5.1
3130259	2018, M 9.5.1 Appendix 3 July 12th TGAI Deficiency Response, DACO: M9.5.1
3130261	2020, Chronic effects of the Microbial Pest Control Product Bb-Protec to *Daphnia magna* in a 21 day reproduction test, DACO: M9.5.2

4.0 Value

PMRA Document Number	Reference
2971971	2018, M 10.2.1 Lab/Growth Chamber Studies, DACO: M10.2.1
2971972	Draganova, S.A. and Simova, S.A., 2010, Suspectibility of *Tetranychus urticae* Koch. (Acari: Tetranychidae) to isolates of entomopathogenic fungus *Beauveria bassiana*. *Pestic. Phytomed.* (Belgrade), 25(1), 2010, 51-57, DACO: M10.2.1

PMRA Document Number	Reference
2971973	Gatarayiha, M. C., Laing, M. D., & Miller, R. M. (2012). Selection of *Beauveria bassiana* strains against the two spotted spider mite, *Tetranychus urticae* Koch in laboratory and greenhouse trials. *African Journal of Microbiology Research*, *6*(11), 2694-2703, DACO: M10.2.1, M10.2.2
2971974	2018, M 10.2.2 Field Trials, DACO: M10.2.2
2971975	2016, M 10.2.2 Appendix 1, DACO: M10.2.2
2971976	2018, M 10.2.2 Appendix 2, DACO: M10.2.2
2971977	2018, M 10.2.2 Appendix 3, DACO: M10.2.2
2971978	2018, M 10.2.2 Appendix 4, DACO: M10.2.2
2971979	2018, M 10.2.2 Appendix 5, DACO: M10.2.2
2971980	2018, M 10.2.2 Appendix 6, DACO: M10.2.2
2971981	2018, M 10.2.2 Appendix 7, DACO: M10.2.2
2971982	2018, M 10.2.2 Appendix 8, DACO: M10.2.2
2971983	2018, M 10.2.2 Appendix 9, DACO: M10.2.2
2971984	2018, M 10.3.1 Phototoxicity and Photopathogenicity, DACO: M10.3.1
2971985	2018, M 10.3.2.1 Effects on the MPCA, DACO: M10.3.2.1
2971986	2018, M 10.3.2.2 Effects on the EP, DACO: M10.3.2.2
2971987	2018, M 10.4.1 Profile of the EP, DACO: M10.4.1
2971988	2018, M 10.4.2 Nature and Economics of the Pest Problem in Canada, DACO: M10.4.2
2971992	2018, M 10.4.3 Current Crop Protection Tools and Practices, DACO: M10.4.3
2971993	2018, M 10.4.4 Compatibility with Current Management Practices including IPM, DACO: M10.4.4
2971994	2018, M 10.4.4 Appendix 1, DACO: M10.4.4
3080680	2020, M 10.1 Value Summary June 25 deficiency response, DACO: M10.1
3080681	2020, M 10.1 Excel Value Summary June 25 Deficiency Response, DACO: M10.1
3080682	2020, M 10.2 Aphids Scientific Rationale June 25 Deficiency Response, DACO: M10.2
3080683	2020, M 10.2 Two Spotted Spider Mite Scientific Rationale June 25 Deficiency Response, DACO: M10.2
3080684	2020, M 10.2 Whitefly Scientific Rationale June 25 Deficiency Response, DACO: M10.2
3080685	Byrne, D. N., & Bellows Jr, T. S. (1991). Whitefly biology. *Annual review of entomology*, *36*(1), 431-457, DACO: M10.2
3080693	Liburd, O.E., Nyoike, T.W. and Razze, J.M., 2015, Biology and management of whiteflies in sustainable field production of cucurbits. *ENY-848, one of a series of the Entomology and Nematology Department, UF/IFAS Extension*. Original publication date January 2008. Revised December 2015, DACO: M10.2
3080695	Osborne, L. S., & Landa, Z. (1992). Biological control of whiteflies with entomopathogenic fungi. *Florida entomologist*, 456-471, DACO: M10.2

PMRA Document Number	Reference
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3080701	2020, 10.2.3 Field Studies June 25 Deficiency Response, DACO: M10.2.2
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3080704	2016, 10.2.3 Appendix 2a, DACO: M10.2.2
3080705	2017, 10.2.3 Appendix 3a, DACO: M10.2.2
3080706	2019, 10.2.3 Appendix 4a, DACO: M10.2.2
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3080709	2020, 10.2.4 Appendix 1, DACO: M10.2.2
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B. Additional information considered

Human and animal health

PMRA Document Number	Reference
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